

Circuits and Signals of the Skin-Associated Lymphoid Tissues (SALT)

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The existence of a specialized set of skin-associated lymphoid tissues (SALT) was proposed several years ago as a means of accounting for (a) epidermotropism of certain malignant lymphocytes, (b) immunocompetent cells within skin, and (c) expression of histocompatibility antigens on cutaneous cells derived from hematopoietic precursors. The proposal was supported by observations of (a) *in vivo* and *in vitro* antigen-presenting potential of epidermal Langerhans cells, (b) discovery of novel bone marrow-derived cells within epidermis, and (c) epidermotropism as a physiologic property of some nonmalignant T lymphocytes. Langerhans cells stand alone among epidermal cells in their capacity to process and present antigens of the intraepidermal compartment in highly immunogenic fashion. However, not all antigens within epidermis lead to hypersensitivity: antigens introduced into Langerhans cell-deficient skin are perceived as tolerogens, and the cellular source of the tolerogenic signal may be Thy-1-positive epidermal cells. An extracutaneous pathway for presentation of epidermally-administered antigen has been described, and is independent of Langerhans cells, genetically determined, and dominant in the face of epidermally derived tolerogenic signals. Only systemic unresponsiveness can mitigate the cutaneous sensitivity produced by the second pathway. Thus, both intra- and extracutaneous forces contribute to the induction and regulation of cutaneous immunity, forces that are integrated via SALT to achieve optimal cutaneous immune protection against pathogens and neoplasms.

review, items 2, 3, and especially 5, which deals with the cellular

and molecular basis of induction and regulation of cutaneous immunity, are explored in detail.

EPIDERMIS CONTAINS CELLULAR ELEMENTS REQUIRED FOR ANTIGEN PROCESSING, PRESENTATION, AND RECOGNITION

By conventional light microscopy, examination of the skin and epidermis reveals that the epidermal compartment, comprised chiefly of epithelial cells, is contaminated by nonepithelial cells, many of which are of mesenchymal origin (i.e., bone marrow-derived). These cells include typical lymphocytes [3], the well-studied epidermal Langerhans cell (LC) [4,5], a novel population of bone marrow-derived cells [6,7] bearing the Thy-1 molecule (albeit lacking in typical T-cell functional properties), and the so-called indeterminant cells. Barker and Bilingham [8] demonstrated more than a decade ago that skin grafts contain immunocompetent lymphocytes within them; one or another of the lymphoid-appearing cells within the epidermis represent candidates for that role. After considerable study, it is apparent that epidermal LC display on their cell surface molecules characteristic of cells involved in antigen presentation (class 2 major histocompatibility complex (MHC) antigens, Fc receptors for immunoglobulin, and the receptor for the third component of complement) [9]. *In vitro* studies indicate that LC possess the functional property of being able to present antigen to primed T lymphocytes [10]. Although it was originally and unexpectedly found that keratinocytes were a source of interleukin-1 (ETAF), it is now clear that epidermal LC also can produce this molecule which plays a crucial role in antigen presentation [11,12].

RECOGNITION OF ANTIGEN BY IMMUNOCOMPETENT LYMPHOCYTES CAN TAKE PLACE WITHIN SKIN

It has been more than 6 years since it was first proposed that a portion of the immune apparatus was specialized and dedicated to provide the skin with immune protection [1]. The acronym SALT was utilized to designate these skin-associated lymphoid tissues. At that time and during the subsequent several years, an increasing body of circumstantial evidence supports the contention that SALT exists. Evidence in favor of SALT includes the following: (1) some but not all lymphoid reticular cells exhibit epidermotropism; (2) epidermis contains cellular elements required for antigen processing, presentation, and recognition; (3) recognition of antigens by immunocompetent lymphocytes can take place within skin; (4) draining regional lymph nodes serve to integrate and direct cutaneous immune responses; and (5) regulation of cutaneous immunity is dictated by elements found within skin itself [2]. In this

The inductive event by which immunocompetent lymphocytes first recognize and respond to the antigen is thought to take place within organized lymphoid tissues such as lymph nodes and spleen. That such a critical event can happen within the skin itself was first demonstrated by Brent and Medawar who inoculated allogeneic lymphocytes into the skin and demonstrated that these cells were able to mount inflammatory reactions as a consequence of *in situ* recognition of alloantigens [13]. Indirect evidence that antigen recognition takes place within skin during induction of contact hypersensitivity (CH) was provided by the studies of Macher and Chase who demonstrated that excision of the cutaneous site of hapten inoculation precluded the development of contact hypersensitivity [14]. Recently by using a method of CH induction that utilizes hapten-derivatized orthotopic skin grafts [15], we demonstrated that genetic elements (encoded by MHC genes) within skin are responsible for restricting the effector specificity of delayed-type hypersensitivity T cells, implying that recognition by T cells was initiated within the skin graft. These findings provide support for the thesis that skin affords a microenvironment in which immunocompetent cells can recognize antigens.

REGULATION OF CUTANEOUS IMMUNITY IS DICTATED BY ELEMENTS WITHIN SKIN

The development of successful protocols for the induction of CH and its study in mice occurred approximately 15 years ago

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Abbreviations:

CH: contact hypersensitivity
EC: epidermal cell(s)
LC: Langerhans cell(s)
MHC: Major histocompatibility complex
SALT: skin-associated lymphoid tissues
TNCB: trinitrochlorobenzene
UVR: UV radiation

TABLE I. *Experimental induction of contact hypersensitivity and unresponsiveness*

a. Maneuvers to Induce Contact Hypersensitivity	Evidence of Down Regulation
Apply epicutaneous hapten: conventional doses (TNCB 5%, DNFB 0.5%, Oxazalone 10%) Graft hapten-derivatized skin (similar doses of hapten to above)	Cyclophosphamide pretreatment causes enhanced response Immobilization of recipient leads to enhanced CH response
Inject hapten s.c.	Method to induce certain suppressor T cells
Inject hapten-derivatized, unfractionated epidermal cells i.v.	Animals are hyporesponsive to re-immunization with hapten
b. Maneuvers That Induce CH Without Down Regulation	
Paint skin with threshold doses of haptens (10–50 μ g TNCB)	
Graft syngeneic skin derivatized with limiting doses of hapten (0.02% DNFB)	
Inject hapten-derivatized, purified Langerhans cells i.v. or s.c.	
Inject hapten-derivatized keratinocytes s.c.	
c. Maneuvers That Induce Down Regulation of CH	
Paint hapten on skin with reduced numbers of normal Langerhans cells	
Paint skin with excess hapten	
Excise site of hapten inoculation (within 24–48 h)	
Inject hapten-derivatized Thy-1 ⁺ epidermal cells i.v.	
d. Maneuvers That Produce Neither Up Nor Down Regulation of CH	
Apply subthreshold doses of hapten (less than 10 μ g TNCB) to skin	
Graft hapten-derivatized allogeneic skin at limiting dose of hapten	
Inject hapten-derivatized keratinocytes i.v.	
Inject hapten-derivatized Thy-1 ⁺ epidermal cells s.c.	

[16]. Since that time, much has been learned about the cellular and molecular bases for the induction and expression of CH. However, CH as a model system in mice differs in several important ways from the more classical models of CH in guinea pigs and in man. In mice, the doses of haptens typically used for CH induction are comparatively high. Moreover, the ability of mice to display CH is relatively short lived, i.e., between 2–3 weeks after induction, mice generally prove to be refractory to challenge with hapten. In addition, induction of suppression and down regulation seems to be part and parcel of every immunizing regimen. This has even led to the inference that every CH-immunizing regimen obligatorily induces concomitant down regulation. In the next section, the validity of this inference will be explored by examining 4 questions concerning methods for inducing CH in mice: (1) What maneuvers regularly induce CH in mice? (2) What maneuvers induce CH *without* evidence of down regulation? (3) What maneuvers primarily induce down regulation of CH? and (4) What maneuvers are immunologically null, i.e., induce neither immunogenic nor tolerogenic signals?

MANEUVERS TO STUDY CONTACT HYPERSENSITIVITY

Evidence bearing on each of these questions is presented in Table I. (1) In Table Ia, 4 different methods for the induction of CH in mice are represented. The right-hand column of the Table indicates that in each instance, there is clear-cut evidence in the immunized animals that down regulation and suppression have been concomitantly induced with CH. Cyclophosphamide-sensitive suppressor cells are revealed when CH is induced in animals by the conventional skin painting technique [17]. Inoculation of hapten directly s.c. is the typical method utilized to generate certain types of suppressor T cells [18,19]. Intravenous inoculation of haptenated epidermal cells (EC), while capable of inducing CH, also induces down regulation revealed when subsequent attempts to immunize these animals by the conventional route fails [20]. Thus, each of these effective methods of inducing CH in mice, also induces one or another form of down regulation.

(2) By altering the experimental protocol, it is possible to produce CH in mice without any detectable signs of a down regulating influence. Three methods described in part in our laboratories over the past several years are listed in Table Ib. Significant levels of CH can be induced *without* evidence of down regulation when threshold doses of antigen are em-

ployed—whether the induction regimen uses direct epicutaneous or the artifice of derivatization of syngeneic orthotopic skin grafts [15]. Most importantly, purified epidermal LC, that have been haptenated in vitro with trinitrophenyl (TNP) and are then inoculated i.v., induce vigorous CH without down regulation [21]. In these instances, animals rendered contact hypersensitive display exaggerated responses to subsequent attempts to reimmunize them with the same hapten.

(3) Several methods have been devised in which suppression of CH dominates the animals' response to hapten challenge (see Table Ic). Originally, we described unresponsiveness as a consequence of painting hapten on cutaneous surfaces with reduced numbers of functionally normal LC [22]. Claman and his colleagues had previously shown that supraoptimal doses of hapten painted epicutaneously lead to suppression of the immune response [23]. Even prior to this observation was that of Macher and Chase who demonstrated that premature excision of the site of hapten inoculation not only interfered with the induction of CH, but rendered these animals specifically unable to respond subsequently to the contactant in question [14]. Most recently, Sabra Sullivan (manuscript in preparation) has used purified, haptenated Thy-1 positive EC to demonstrate that as few as 6000 of these cells inoculated i.v. produce specific and profound unresponsiveness without evidence of CH.

(4) Yet not all experiences with hapten lead to up or down signals (see Table Id). For example, doses of epicutaneously applied hapten have now been identified which are simply below the threshold of immunologic recognition [24]. Sullivan et al have found that 1 μ g of trinitrochlorobenzene (TNCB) placed on the epidermis fails to impact the immune system at all. In the past, we have reported that hapten-derivatized, orthotopically grafted, allogeneic skin also fails either to sensitize or render unresponsive recipient mice [15]. This was true if the amount of hapten applied to the allogeneic graft was at the threshold which allowed haptenated, syngeneic grafts to induce CH. Finally, Sullivan has recently shown that haptenated, purified keratinocytes from mouse skin, when inoculated i.v. at doses of 6000 per recipient, fail to induce either sensitivity or unresponsiveness, although this same number of cells injected s.c. induces typical CH.

This spectrum of results in which on the one hand CH without down regulation can be induced while on the other hand specific (virtually uncontaminated) unresponsiveness can be induced suggests that the antigen-specific signals that emerge from the skin during the induction of CH, and that lead

to sensitization as well as unresponsiveness, are separate and discrete entities. To rephrase this suggestion in cellular terms, we propose that LC are the epidermal source of an unambiguous, immunogenic signal following haptenization of the epidermis. By contrast, Thy-1-positive epidermal cells are the singular source of a different signal whose effect is the induction of down regulation of CH. Keratinocytes, at least in the doses in which we have examined them, are not the source of either of these signals.

CHARACTERISTICS OF EPIDERMALLY-DERIVED SIGNALS

A rudimentary attempt can be made to characterize the Langerhans cell-derived signal, based on several lines of experimental evidence: this signal can be delivered by haptenated LC that are injected s.c., by hapten-derivatized syngeneic skin grafts, and even by hapten-derivatized grafts rendered nonviable by heating to 60°C for 1 h [15]. However, the signal is mitigated by pretreatment of the skin painting site with UV radiation, and the signal from haptenated allogeneic skin grafts is immunologically uninterpretable or recognizable. These data suggest that the immunogenic signal from the epidermis contains the haptenic determinant, restricting elements of the H-2 complex (either class 1 or class 2 molecules), is derived from a UV-sensitive source, (Langerhans cell), but need not be present on a viable cell in order to be effective (Table II).

The nature of the down-regulating signal from the skin is much less well understood (Table II). The following characteristics seem relevant: haptenated Thy-1 cells delivered i.v. induce down regulation, while the same cells injected s.c. produce no effect whatever. In addition, the I-J dependent signal derived from the skin (described by Granstein and Green [25]) appears to be insensitive to UV radiation (UVR). Bergstresser has demonstrated that Thy-1-positive cells rarely survive and persist in the epidermis of orthotopically-grafted skin [26]. Haptenated, orthotopic syngeneic skin grafts at limiting doses of hapten are unable to generate down regulation. We suspect therefore that the down-regulating signal of the epidermis contains the haptenic moiety, is derived from the Thy-1⁺ EC which is impervious to exposure to UVR; the signal is recognizable by components of the immune system which are located systemically (spleen) rather than locally (draining lymph nodes).

FACTORS THAT DETERMINE SENSITIVITY OR UNRESPONSIVENESS

Based on these fragmentary pieces of experimental evidence, it is possible (albeit risky) to formulate a hypothesis to account for the paradox that CH can be induced regularly both in the laboratory and the clinic, yet the epidermis is a source of countermanding immunologic signals following epicutaneous application of hapten. This formulation considers two factors

to be of overriding importance in determining whether, and if, CH or down regulation follows hapten exposure. The first is the dose of epicutaneously applied hapten. With respect to the dose response curve to TNCB described in several strains of mice by Sabra Sullivan, it was found that below the threshold of 10 µg of TNCB, there was no effect upon the immune system [24]. In the narrow dose range above the threshold dose (10–50 µg), CH was induced without evidence of down regulation. However, as the dose was raised further beyond that point, in a strain-dependent fashion, an ever increasing component of down regulation was induced. However, at no dose, including the highest 14,000 µg (28% TNCB in carrier), did mice fail to develop detectable CH. We believe this means that at threshold doses of epicutaneously applied hapten, only LC are able to prepare a hapten-specific signal—and that signal is exclusively immunogenic. Higher doses of hapten appear to be required for Thy-1-positive EC to prepare their down-regulating signal. At even higher doses, unconjugated hapten escapes the epidermis and interacts directly with components of the immune system further upstream, producing a poorly understood array of effects.

The second factor we feel to be of importance in determining whether up- or down-regulating signals dominate in CH, is the relationship between epidermally-derived signals, the constituents of SALT, and the interaction of SALT with the systemic immune apparatus. In Table III are listed the immune effects of the several purified populations of epidermal cells we have prepared, haptenated, and then inoculated by s.c. and i.v. routes. It was observed that LC, when haptenated, deliver an immunogenic signal by both routes. By contrast, Thy-1⁺ cells when hapten-derivatized and inoculated i.v. induce only down regulation; significantly, they make no detectable impact when placed s.c. Finally, keratinocytes that have been hapten-derivatized are able to immunize when injected s.c., but are immunologically “null” when injected i.v. We interpret these results to mean that lymph nodes draining the cutaneous site of hapten application are able directly to receive and transduce the Langerhans cell-derived signal, however these same nodes appear to be incapable of receiving and/or transducing the epidermally-derived down-regulating signal. Instead, the receiver for this signal is at a more central site (which we presume to be the spleen) that is responsible for successful induction of down regulation. Since intravenously-delivered, haptenated LC induce CH without suppression, receivers of this immunogenic epidermally-derived signal must also reside at central systemic (splenic) sites.

CIRCUITS OF RECOGNITION AND REGULATION IN CH

In the induction of CH and its regulation, one can construct hypothetical pathways or circuits by which antigen is processed, presented, and recognized. In Table IV, several nonmutually exclusive circuits are described. We believe that under normal circumstances, the dose of epicutaneously applied hapten dictates whether CH, with or without down-regulating component, will take place. At the lowest, effective dose of hapten (for example 25 µg TNCB applied epicutaneously), circuit 1 would apply. At higher doses, the second circuit would also be activated, thereby enlisting 2 up-regulating processes to produce the contact hypersensitive state. Circuit three, which is revealed in the artificial circumstances of our experiments, indicates that the LC-derived signal is a dominant one, and mediates its effect even when the draining lymph node is bypassed. Circuits 4 and 5 describe the process by which epidermally prepared down-regulating signals, as well as i.v. delivered hapten, influence the development of CH. In both instances, the site of recognition of antigen, which results in the generation of suppression, is centrally placed, presumably in the spleen. Thus, since in normal circumstances epicutaneously administered hapten reaches the draining lymph nodes prior to the

TABLE II. *Characteristics of epidermally-derived signals by contact hypersensitivity*

Immunogenic	Tolerogenic
Hapten-specific	Hapten-specific
MHC-restricting elements	(?) I-J restricting elements
Derived from Langerhans cells	Derived from Thy-1 ⁺ epidermal cells
UVR-sensitive	UVR-resistant
Survive grafting procedure	Destroyed during grafting procedure

TABLE III. *Immune effects of hapten-derivatized epidermal cells are dependent upon route of inoculation*

Type of cell	Route of inoculation	
	Subcutaneous	Intravenous
Langerhans cells	↑	↓
Thy-1 ⁺ epidermal cells	—	↑
Keratinocytes	↑	—

TABLE IV. *Circuits for antigen recognition in contact hypersensitivity*

Form of antigen	Presenting cell	Site of recognition	Effect
1. Free hapten	Langerhans cell	Skin and/or lymph node	CH
2. Haptenated keratinocyte	Interdigitating Cell	Lymph node	CH
3. Haptenated Langerhans cells	Interdigitating or follicular dendritic cells	Lymph node	CH
4. Free hapten	Thy-1 ⁺ Cell	Spleen	Unresponsiveness
4a. Free hapten	Thy-1 ⁺ Cell	Lymph node	Unresponsiveness
5. Free hapten or haptenated nondendritic cells	??? (Intravenous presentation)	Spleen	Unresponsiveness

(4a. Proposed circuit in UVR-sensitive mice).

central compartment (the spleen), the first signal produced, i.e., the immunogenic signal derived predominantly from LC, dominates the early immune response, being expressed as CH.

We have previously reported that LC of all mice are susceptible to the deleterious effects of UVR; however, the induction of unresponsiveness following UVR treatment and local application of hapten is the property of only some genetically defined strains of mice [2]. We have proposed that mice who are not susceptible to the UV effect possess two pathways for the effective processing of epicutaneously applied hapten, only one of which is LC-dependent (and UVR-sensitive). We presume that the antigen-presenting cells of the second pathway reside in the draining regional lymph nodes, perhaps the interdigitating cells of circuit 2 (Table IV). It is pertinent in this regard that Glass et al* have recently discovered that suppressor cells are induced in all strains of mice following hapten application to UVR pretreated skin, irrespective of whether these strains prove to be unresponsive in vivo or not. This finding corroborates the observations of Granstein et al [25] that the I-J dependent, epidermally-derived suppressor moiety is UV insensitive. This raises the possibility that the so-called second pathway of antigen presentation is not missing in UV-susceptible mice. Rather, in these mice the facility able to receive epidermally-derived down regulating signals may not be restricted to the spleen, but may also reside within draining regional lymph nodes (circuit 4a, Table IV). By contrast, in UV-resistant mice, the receiver of down regulating signals is placed exclusively at the systemic (spleen) site. Thus, when (by experimental manipulation or by a clinically relevant environmental effect) the epidermis is robbed of its LC-dependent function, the factor which will determine whether immune protection is afforded the skin is likely to be the genetically determined manner in which SALT is deployed, i.e., whether the receiver for down-regulating signals is located regionally or centrally. Although much remains to be learned about the signals and circuits of this system, it is already possible to imagine how the system could be manipulated for therapeutic gain.

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